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Remarks:
The sequence listing, which is published as annex to the application documents, was filed after the date of filing. The applicant has declared that it does not include matter which goes beyond the content of the application as filed.

(54) Mixture comprising an inhibitor or suppressor of a gene and a molecule binding to an expression product of that gene

(57) A mixture comprising at least one inhibitor or suppressor of the expression of a gene and at least one molecule binding to an expression product of said gene.

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Description

[0001] Classically, molecules including drugs, used to modulate biological functions through gene products and their derivatives - like e.g. glycosylated, phosphorylated or otherwise modified gene products, have either stimulated or inhibited gene products and/or their derivatives. Stimulation or inhibition was achieved e.g. by use of agonists or antagonists, including small molecular weight molecules, peptides, antibodies etc. Well known examples are H2-antagonists, and β-blockers or antibodies binding to HER2 protein or to immunomodulating receptors as well as hormone receptor binding molecules.

[0002] Binding can also be achieved by nucleic add derivatives like aptamers and spiegelmers.

[0003] More recently the inhibition of gene products was also used by inhibiting their expression through antisense, ribozymes, triple helix binders etc. Examples are the Inhibition of expression of neurotransmitter receptors In brain or inhibition of cell growth regulating proteins, cytokines and growth factors.

[0004] Both approaches, either inhibition of gene products by binding of molecules to the gene products and their derivatives or alternatively inhibition of expression have been used for a large variety of gene products and their derivatives.

[0005] The invention pertains to a mixture comprising at least one inhibitor or suppressor of the expression of a gene and at least one molecule binding to an expression product of said gene.

[0006] Surprisingly, this combination shows a supra-additive effect. "Supra-additive" Is defined as a effectiveness of a mixture that Is at least 20 %, preferably more than 50 %, more preferably more than 100 % better than the sum of the effect of the single compounds of the mixture. This can be tested in any *in vitro* or *in vivo* system, which a) expresses the respective gene and b) the expression of the gene has a measurable effect in the system.

[0007] Advantages are lower doses and/or higher efficiency compared to each individual approach.

[0008] Preferably, the at least one Inhibitor or suppressor is an nucleic acid molecule or derivative thereof. The at least one nucleic acid molecule is preferably an oligonucleotide, an antisense oligonucleotide and/or a ribozyme inhibiting or interfering with the expression of a gene which plays a role in a patho-physiological event.

[0009] Derivatives of gene products are e.g. posttranscritptionally or postranslationally modified gene products e.g. RNA or proteins which have undergone editing or chemical modification e.g. by methylation, phosphorylation, glycosylation etc.

[0010] According to the invention it is useful to integrate the antisense and/or ribozyme molecule into a DNA delivery system. The DNA delivery system comprises viral or non-viral vectors or both and additionally anionic lipids, catlonic lipids, non-cationic lipids or mixtures thereof.

[0011] Preferably, the antisense and/or ribozyme molecule is modified at one or more of the sugar moieties, the bases and/or the internucleotide linkages, e.g. the phosphate bonds. For example, the modification of the oligonuclcotides, ribozymes and/or nucleic acids comprises modifications such as phosphorothloate (S-ODN) internucleotide linkages, methylphosphonate internucleotide linkages, phosphoramidate linkages, peptide linkages, 2'-O-alkyl modifications of the sugar, in particular methyl, ethyl, propyl, butyl and the like, 2'-methoxyethoxy modifications of the sugar and/or modifications of the bases. The various modifications may be combined in an oligo- or polynucleotid.

[0012] The antisense and/or ribozyme molecule can also be modified by coupling it to an enhancer of uptake and/or inhibitory activity.

[0013] In a further preferred embodiment of the invention the nucleic acid molecules are coupled to or mixed with folic add, hormones, steroid hormones such as oestrogene, progesterone, corticosteroids, mineral corticoids, peptides, proteoglycans, glycolipids, phospholipids and derivatives thereof.

[0014] In a very preferred embodiment, the nucleic acid molecule is selected from nucleic acid molecules comprising one or more of the following nucleotide sequences:

GTA GTA CAC GAT GG (Seq. ID. No. 1)

CTG ATG TGT TGA AGA ACA (Seq. ID. No. 2)

CTC TGA TGT GTT GAA G (Seq. ID. No. 3)

CGG CAT GTC TAT TTT GTA (Seq. ID. No. 4)

GCT TTC ACC AAA TTG GAA GC (Seq. ID. No. 5)

CTG GCT TTT GGG TT (Seq. ID. No. 6)

GCT GTT GAC TGC CC (Seq. ID. No. 7)

CCC AGT ATT ACT GC (Seq. ID. No. 8)

GGT TGA AGC CAT TG (Seq. ID. No. 9)

GCC GCT CAA TCT TCA TC (Seq. ID. No. 10)

GAA CAG TTC GTC CAT G (Seq. ID. No. 11)

CCA GAG TTT CGG TTC (Seq. ID. No. 12)

CTA GGA CTG GGA CAG (Seq. ID. No. 13)

CAT CTT CTG CCA TTC (Seq. ID. No. 14)

CGT AGG TGG TGC TG (Seq. ID. No. 15)

GTG TTT TCC CAC CAG (Seq. ID. No. 16)

GGT TTT GGT TCA CTA G (Seq. ID. No. 17)

[0015] In a further embodiment, the Inhibitor or supressor is a peptide, protein and/or low molecular weight substance, which Is able to bind to DNA or RNA coding for the gene, thus Inhibiting or suppressing expression of the gene. Suitable proteins also comprise antibodies and antibody fragments.

[0016] The mixture of the Invention comprises preferably as the at least one molecule binding to the expression product of the gene an antibody, antibody fragment, such as a F_{ab} fragment, single chain antibody or combinations thereof. The antibody, antibody fragment, such as a F_{ab} fragment, single chain antibody or combinations thereof are e.g. obtainable by screening of antibody libraries and testing the expression products for binding to an expression product of the gene.

[0017] The at least one molecule binding to an expression product of the gene is preferably a peptide and/or protein. The peptide and/or protein is e.g. obtainable by screening an expression library and testing the expression products for binding to an expression product of the gene.

[0018] The synthetic peptide and/or protein may also be obtained by screening randomly synthesised peptides and/or polypeptides for binding to an expression product of the gene.

[0019] In another embodiment, the mixture of the invention comprises a low molecular weight molecule binding to an expression product of the gene. In particular, the low molecular weight molecule is obtainable by using combinatorial chemistry and testing the products for binding to an expression product of the gene.

[0020] The molecule or factor binding to an expression product of the gene may also be DNA or RNA molecule or a derivative thereof Including aptamers and/or splegelmers that bind to the expression product to the gene.

[0021] In a preferred embodiment of the Invention, the gene is selected from the group consisting of TGF-β, erbB-2, MIA, c-jun, junB, c-fos, VCAM, NF-kappaB p65, NF-kappa B p50, ICAM, VEGF and NF-kB 2.

[0022] The invention pertains as well to oligonudeotides having the sequences Seq. ID, No 1 to 17.

[0023] A medicament comprising the mixture of the Invention is also subject matter of the present invention.

[0024] The invention further concerns a method of using a mixture comprising at least one supressor or inhibitor of the expression of a gene and at least one molecule or factor binding to an expression product of said gene for treating tumors, immune disorders, or Improving organ or cell transplantation.

[0025] The invention furthermore pertains to a method of treating tumors, immune disorders, or improving organ or cell transplantation by administration of an effective amount of a combination of at least one molecule or factor suppressing or inhibiting an expression of a gene and at least one factor binding to an expression product of said gene whereby inhibition of tumor growth, improvement of organ or cell transplantation, enhancement or inhibition of immune response is enhanced in a supra-additive manner.

[0026] The mixture of the present invention is also useful in Drug Target Validation, i.e. to identify genes that are relevant for a certain pathological state by testing the effect of the mixture of the present invention on a cell system or organism.

[0027] Fig. 1 shows a strongly supra-additive effect of a combination of both the blocking of the gene with the antisense molecule combined with the neutralising antibody.

Examples

Example 1

[0028] To study the effects of neutralising antibody against TGF-β2 as an Inhibitor of the gene product, antisense oligonucleotides against TGF-β2 as an inhibitor of gene expression and a combination of the two, upon immune response activity of cytotoxic T-lymphocytes (CTL) and Lymphokine activated killer cells (LAK cells) on tumor cells, a CARE-LASS assay has been employed (Lichtenfels, R., Biddison, W.E.1 Schulz, H.1 Voyt, A.B. and R. Martin. CARE-LASS (calcein-release assay), an improved fluorescence based test system to measure cytotoxic lymphocyte activity. J. Immunol. Meth., 172: 227-239,1994).

Generation of LAK cells and CTLs

[0029] Peripheral blood mononuclear cells (PBMC) were Isolated from venous blood of healthy donors by standard Flcoll-Hypaque gradient centrifugation, as described previously. Briefly, heparinized blood was mixed with equal volumes of complete medium (RMPI 1640 medium supplemented with 10% (v/v) fetal calf serum and 1 mM L-Glutamine) and layered onto Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradients. After centrifugation at 400g for 30 min at room temperature, PBMCs banded at the plasma-Ficoll interface were removed, washed tree times and resuspended in complete medium. Cell viability, as determined by Trypan blue exclusion, was > 97%. Lymphocytes were activated by treatment with 10 U/ml IL-1 alpha and 100 U/ml IL-2.

[0030] At the day of the assay, glioma tumor cells were harvested, washed twice in 5% FCS/PBS solution and resuspended at 10 Mio cells/ml in 5% FCS/PBS. Calcein-AM (Molecular Probes, USA) was added to a final concentration of 25 μM. The cells were labeled for 30 min at 37° C then washed twice in 5% FCS/PBS, adjusted to 1 Mio cells / ml and loaded into 96-well U-shaped microtiter plates at the final concentration of 0.1 Mio / 100 μL / 1 well (Nunc, Denmark).

[0031] Either

- antisense phosphorothioate TGF-β2 oligonucleotides (f.c. 2-5 μM),
  or
- neutralising antibody against TGF-β2 (f.c. 100 mg/ml)
  or
- a combination of the two
  or
- neither of the two

  were added as indicated in the Figure.

[0032] Measure of cytotoxic activity of effector cells (E) on the target cells (T):

[0033] To measure cytotoxic activity of effector cells, wells were loaded with 100 μM of CTL and LAK cells (E) to produce the desired E : T ratios of 1:20.

Spontaneous release of Calcein

[0034] To measure spontaneous release and total release of calcein, wells were preloaded with 100 µl 5% FCS/PBS or 100 µL lysis buffer (50 nM sodium borate, 0.1% Triton x 100, pH 9.0) respectively. After incubating the plate for 4 h at 37° C the supernatants (50 µL) were transferred into new wells and measured using an automated fluorescence scanner(Titertek Fluoroskan II, Germany). The cytotoxicity was determined from the following equation:

$$\frac{\text{F/CTL assay - F spontaneous release}}{\text{F total lysis - F spontanous release}} \times 100 = \%\ \text{cytotoxicity}$$

Example 2

[0035] Hematopoletic Stem cells were collected by apheresis after mobilisation from bone marrow with a daily dose of 10 µg/kg/day of rhG-CSF for 5 days or from cord blood (cord blood stem cells) and enrichment of CD34+ cells achieved with immunopurification.

[0036] The multipotent progenitor fraction of both, bone marrow derived and cord blood derived stem cells, are of critical relevance for clinical application. A problem of current stem cell transplantation is the low number of stem cells generated by optimised cytokine cocktails. Furthermore a critical problem for long term success Is the quiescence and maturation of multipotent progenitor fraction by current treatment with cytokine cocktails.

[0037] Both the number of cells and the proliferative capacity of bone marrow stem cells can be Improved by treatment with TGF-β1 inactivating antibodies or with TGF-β1 antisense oligonucleotides.

[0038] Surprisingly a combination of both, TGF-β1 inactivating antibodies and TGF-β1 antisense oligonucleotides had a strongly supra-additive effect.

[0039] Bone marrow derived and cord blood derived stem cells were treated with either

- antisense TGF-β1 oligonucleotides,
  or
- neutralising antibody against TGF-β1
  or

a combination of the two
or
neither of the two (controls).

[0040] The number of multipotent proliferating progenitor cells was increased by 85 % through treatment with TGF-β1 antisense oligonucleotides compared to controls.

[0041] The number of multipotent proliferating progenitor cells was increased by 63 % through treatment with TGF-β1 neutralising antibody compared to controls.

[0042] The number of multipotent proliferating progenitor cells was increased by more than 350 % through treatment with a combination of both, TGF-β1 inactivating antibodies and TGF-β1 antisense oligonucleotides compared to controls.

Example 3

[0043] A combination of both, TGF-β1 binding peptide and TGF-β1 antisense oligonucleotides had a similarly strongly supra-additive effect on the proliferation of multipotent proliferating hematopoletic progenitor cells.

[0044] The number of multipotent proliferating progenitor cells was increased by 85 % through treatment with TGF-β1 antisense oligonucleotides compared to controls.

[0045] The number of multipotent proliferating progenitor cells was increased by 57 % through treatment with TGF-β1 binding peptide compared to controls.

[0046] The number of multipotent proliferating progenitor cells was Increased by more than 3-fold through treatment with a combination of both, TGF-β1 binding peptide and TGF-β1 antisense oligonucleotides compared to controls.

Example 4

[0047] The c-*erb*B-2 gene (also called p185, *HER-2* or *neu)* is amplified and/or overexpressed in 30-45% of human mammary carcinomas, and in up to 50% in pancreas carcinomas, ovarina cancer, gastric carcinomas, non-small-cell lung cancer, oral squamous cell carcinomas.

[0048] An inactivating antibody for cerbB-2 or HER2 with the trade name Herceptin® has been used for treatment

of breast cancer patients. Clinical studies initially showed good therapeutic potential, while current clinical studies give controversial results. We found that a combination of an inhibitor of cerbB-2 gene expression with a molecule binding to the cerbB-2 gene product strongly enhanced the effect of each molecule alone.

[0049] Ovarian carcinoma cells and pancreas carcinoma cells were treated either with either

- antisense c-erbB-2 oligonucleotides,
  or
- neutralising antibody against c-erbB-2
  or
  a combination of the two
  or
  neither of the two.

[0050] Inhibition of tumor cell proliferation was between 18% and 31% with antisense c-erbB-2 oligonucleotides, between 13 and 34% with antibodies, but by more than 85% with a combination of the two.

Example 5

[0051] Inhibition of endogenous MIA synthesis by a transfecting vector expressing the antisense sequence:

GGCAGGGCCAGCGGTAGGCTGAGCTCACTGGCAGTAGAAATCCCATTTGT

CTGTCTTCACATCGACTTTGCCAGGTTTCAGGGTCTGGTCCTCTCGGACA

ATGCTACTGGGGAAATAGCCCAGGCGAGCAGCCAGATCTCCATAGTAATC

TCCCTGAACGCTGCCTCCCCAGAAGAGCCGCCCACGGCCCTTCAGCTTGG

AGAAGACATACACCACTTGGCCCCGGTGAATGGTCAGGAATCGGCAGTCG

GGGGCCATGTAGTCCTGAAGGGCCACAGCCATGGAGATAGGGTGGCTGCA

CTCCTGGTCCGCACACAGCTTCCGGTCAGCCAGCTTGGGCATAGGACCAC

CCCTGACACCAGGTCCGGAGAAGGCAGACAGCAAGATGATGACACCAAGG

CACACCAGGGACCGGGCCATCGTGGACTGTGAGCAAGAGAGTGAGCAAGG

GGGTGCTGG

or parts of this sequence in human, MIA-secreting melanoma as well as breast cancer cell lines reduced their migration activity, as well as increasing their adhesion to matrices, both suggesting a strong inhibitory effect of MIA inhibitors an tumor invasion and metastasis.

[0052] Supra-additive effects were achieved with a combination of a MIA binding peptide with a transfecting vector expressing the above antisense sequence on reduction of their migration activity, as well as increasing their adhesion to matrices.

Example 6

[0053] Supra-additive inhibition can also be achieved by combining a transcription factor or Its binding domain, binding to a regulatory sequence of receptors, enzymes, transcription factors, cell adhesion molecules, cytokines or growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrins genes with a molecule of small molecular weight, e.g. derived by combinatorial chemistry, binding to receptors, enzymes, transcription factors, cell adhesion molecules, cytokines or growth factors, such as TGE-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrins.

Example 7

[0054] Supra-additive inhibition can also be achieved by combining a peptide or a protein, binding to the mRNAs transcribed from receptors, enzymes; transcription factors, cell adhesion molecules, cytokines, or growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrin genes with a small molecule binding to receptors, enzymes, transcription factors, cell adhesion molecules, cytokines, or growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrins.

Example 8

[0055] Supra-additive inhibition can also be achieved by combining peptide, a protein e.g. a transcription factor or its binding domain, binding to a regulatory sequence of receptors, enzymes, transcription factors, cell adhesion molecules, cytokines growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrin genes with a Spiegelmer binding to receptors, enzymes, transcription factors, cell adhesion molecules, cytokines growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrins.

Example 9

[0056] Supra-additive Inhibition can also be achieved by combining a transcription factor or its binding domain, binding to a regulatory sequence of receptors, enzymes, transcription factors, cell adhesion molecules, cytokines or growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrin genes with peptides binding to receptors, enzymes, transcription factors, cell adhesion molecules, cytokines or growth factors, such as TGF-β, MIA, VCAM, ICAM, c-jun, junB, NF-kappaB, VEGF or integrins.

[0057] The mixtures of examples 6 to 9 are especially useful for neuronal stem cell expansion.

Example 10

[0058] Supra-additive effects can also be achieved by combining an inbibitor of c-jun expression with a molecule binding the c-jun gene product or derivative thereof. Such mixtures are useful for the protection of neurons to ischaemia, hypoxia, degeneration or overstimulation.

SEQUENZPROTOKOLL

<110> Biognostik Gesellschaft für biomolekulare Diagnost

<120> Mixture comprising an inhibitor or suppressor of a gene and a molecule binding to an expression product of that gene

<130> EP00105190.3 Biognostik

<140> 001058190.3
<141> 2000-03-11

<160> 18

<170> PatentIn Ver. 2.1

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Claims

1. A mixture comprising at least one inhibitor or suppressor of the expression of a gene and at least one molecule binding to an expression product of said gene.

2. The mixture of claim 1 wherein the at least one inhibitor or suppressor is a nucleic acid molecule or a derivative thereof.

3. The mixture of claim 2 wherein the nudeic acid molecule is an oligonucleotide, an antisense oligonucleotide and/or a ribozyme molecule.

4. The mixture of claim 3 wherein the oligonucleotide, the antisense oligonucleotide and/or the ribozyme molecule is integrated into a DNA delivery system comprising viral and/or non-viral vectors together with lipids selected from the group of anionic lipids, cationic lipids, non-cationic lipids and mixtures thereof.

5. The mixture of claim 3 wherein the antisense oligonucleotide and/or ribozyme molecule is modified at one or more of the sugar moieties, the bases and/or the internucleotide linkages and/or by coupling the antisense and/or ribozyme molecule to an enhancer of uptake and/or inhibitory activity.

6. The mixture of claim 2 wherein the oligonucleotide has any one of the nucleotide sequences Seq. Id. No 1 to 17.

7. The mixture of claim 1, wherein the at least one inhibitor or supressor is a peptide and/or a protein.

8. The mixture of any one of claims 1 to 7 wherein the at least one molecule binding to the expression product of the gene is an antibody, antibody fragment, such as a F_{ab} fragment, single chain antibody or combinations thereof.

9. The mixture according to claim 8 wherein the antibody, antibody fragment, such as a F_{ab} fragment, single chain antibody or combinations thereof is derived by screening antibody libraries and testing the expression products for binding to an expression product of the gene.

10. The mixture of any one of claims 1 to 7 wherein the at least one molecule binding to an expression product of the gene is a peptide and/or protein.

11. The mixture according to claim 10 wherein the peptide and/or protein is derived by screening an expression library and testing the expression products for binding to an expression product of the gene or is derived by screening randomly synthesised peptides and/or proteins (?) for binding to an expression product of the gene.

12. The mixture according to claim 1, wherein the molecule or factor binding to an expression product of the gene is a low molecular weight molecule.

13. The mixture according to claim 12 wherein the low molecular weight molecule inhibitor Is derived using combinatorial chemistry and testing the products for binding to an expression product of the gene.

14. The mixture according to claim 1, wherein the molecule binding to an expression product of the gene is a DNA or

RNA molecule or a derivative thereof including aptamers and/or spiegelmers.

15. The mixture of any one of claims 1 to 14 wherein the gene is a gene selected from the group consisting of TGF-β, erbB-2, MIA, c-jun, junB, c-fos, VCAM, NF-kappaB p65, NF-kappa B p50, ICAM, VEGF and NF-kB 2.

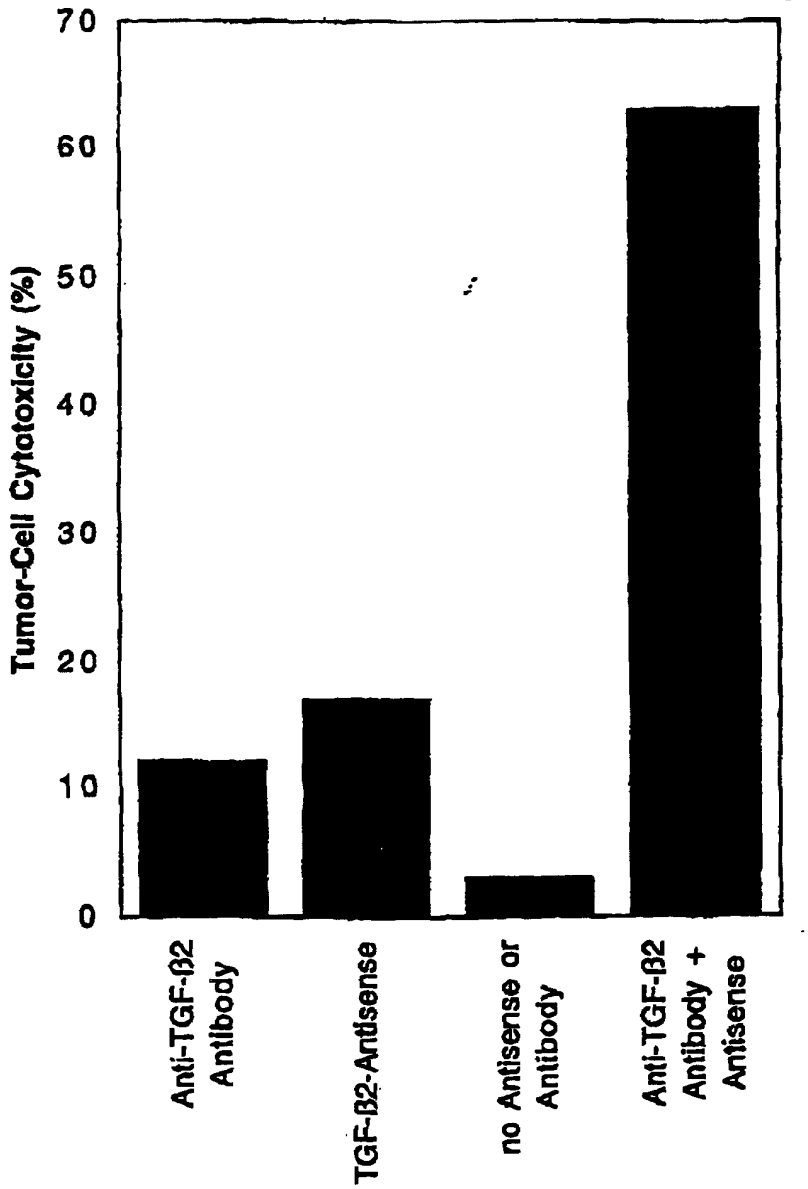
16. Oligonucleotides having the sequences Seq. ID. No 1 to 17.

17. A medicament comprising the mixture of any one of claims 1 to 15.

18. Use of the mixture according to claims 1 to 15 the preparation of a medicament for treating tumors, Immune disorders, or improving organ or cell transplantation or cell expansion.

19. Use according to claim 18 wherein inhibition of tumor growth, improvement of organ or cell transplantation, cell expansion, enhancement or inhibition of immune response is enhanced in a supra-additive manner.

Lysis of tumor-cells: LAK-Cytotoxicty.
Ratio of glioma-cells/LAK-Cells: 1:20



European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 00 10 5190

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	US 5 891 858 A (RUBENSTEIN M) 6 April 1999 (1999-04-06) * the whole document *	1-5, 7-15, 17-19	A61K31/7088 A61K48/00 A61K39/395 C12N15/11 A61P35/00 A61P37/00
A	WO 99 50411 A (ROCHE DIAGNOSTICS) 7 October 1999 (1999-10-07) * the whole document *	1-19	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K
C12N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	22 December 2000	Moreau, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 00 10 5190

Claim(s) searched completely:
16

Claim(s) searched incompletely:
1-15, 17-19

Reason for the limitation of the search:

Present claims 1-15 and 17-19 relate to a compound defined by reference to a desirable characteristic or property, namely the inhibition or suppression of the expression of a gene.
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds defined in the examples 1, 2, 3 and 4.

Present claims 2-5, and 7-15 relate to an extremely large number of possible compounds. Support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the antisenses compoundsindicated in the examples and closely related homologous compounds mentioned in the examples 1, 2, 3 and 4 of the description.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 10 5190

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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22-12-2000

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